Late-onset retinal degeneration pathology de to mutations in CTRP5 is mediated through HTRA1

Figure 1:

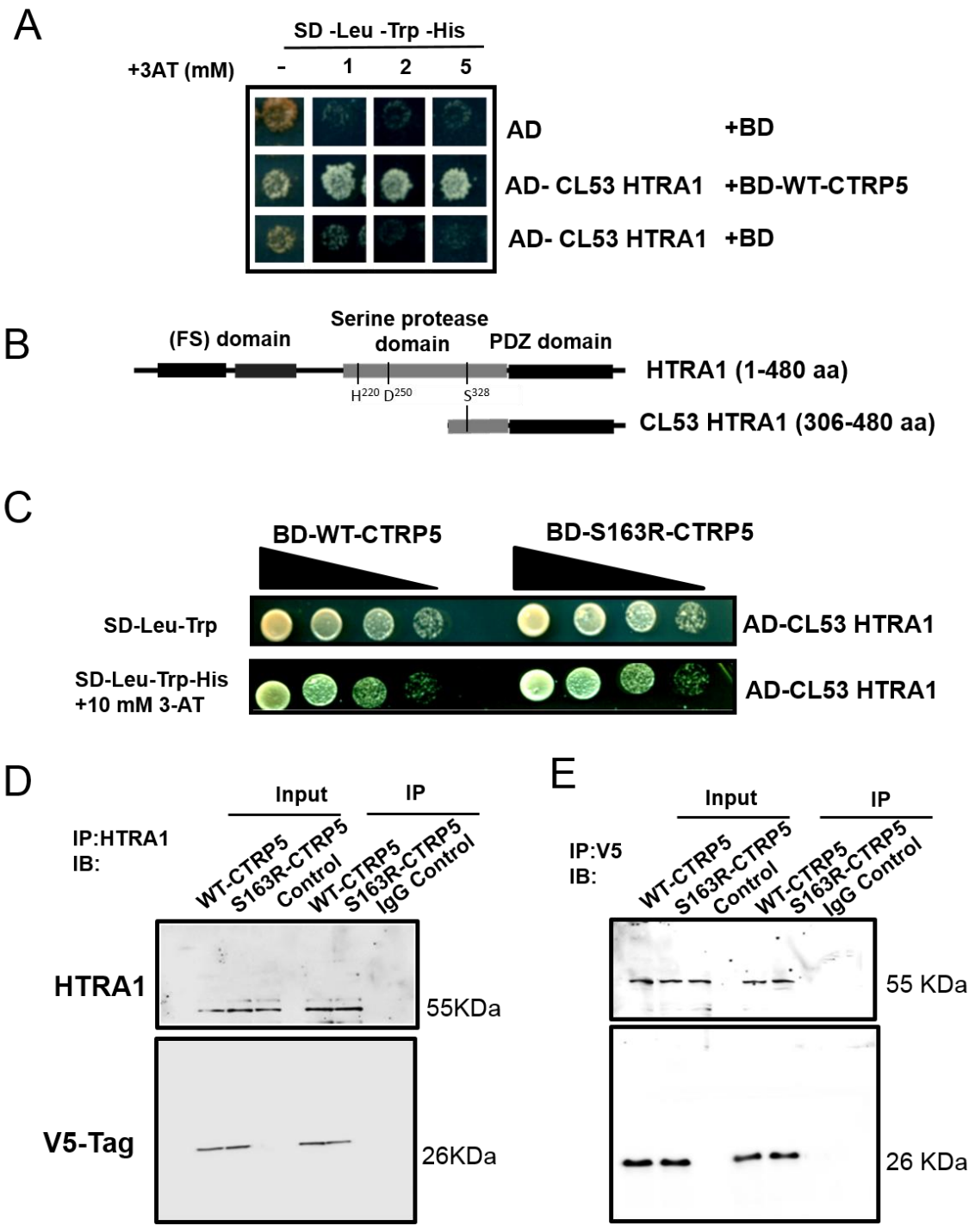

A
SD -Leu -Trp -His
+3AT (mM)
-
1
2
5
AD
+BD
AD- CL53 HTRA1
+BD-WT-CTRP5
AD- CL53 HTRA1
+BD
B
(FS) domain
Serine protease domain
PDZ domain
H220 D250
S328
HTRA1 (1-480 aa)
CL53 HTRA1 (306-480 aa)
C
BD-WT-CTRP5
BD-S163R-CTRP5
SD-Leu-Trp
AD-CL53 HTRA1
SD-Leu-Trp-His
+10 mM 3-AT
AD-CL53 HTRA1
D
Input
IP
IP:HTRA1
IB:
WT-CTRP5
S163R-CTRP5
Control
WT-CTRP5
S163R-CTRP5
IgG Control
HTRA1
55KDa
V5-Tag
26KDa
E
Input
IP
IP:V5
IB:
WT-CTRP5
S163R-CTRP5
Control
WT-CTRP5
S163R-CTRP5
IgG Control
55 KDa
26 KDa

Figure 2:

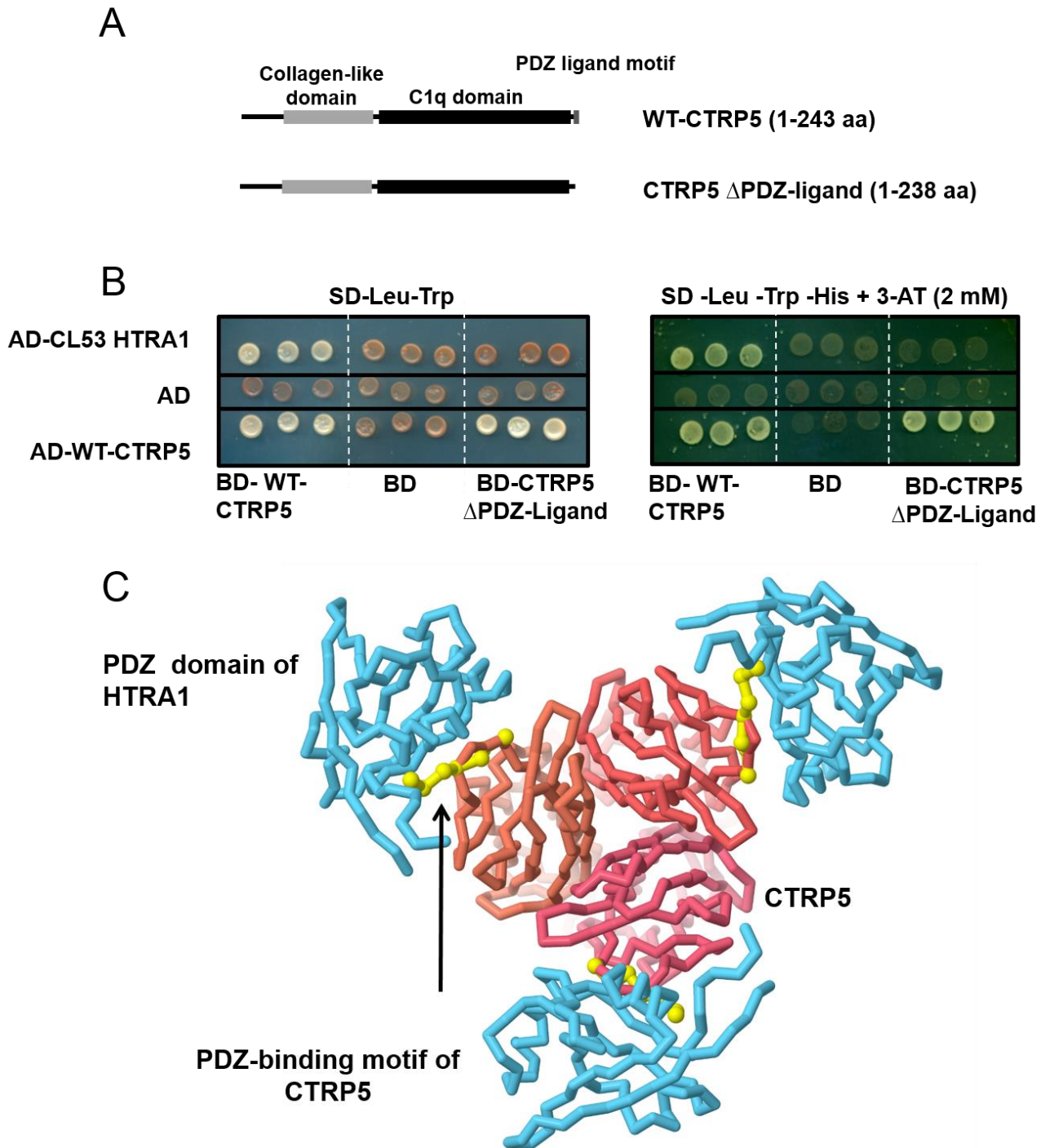
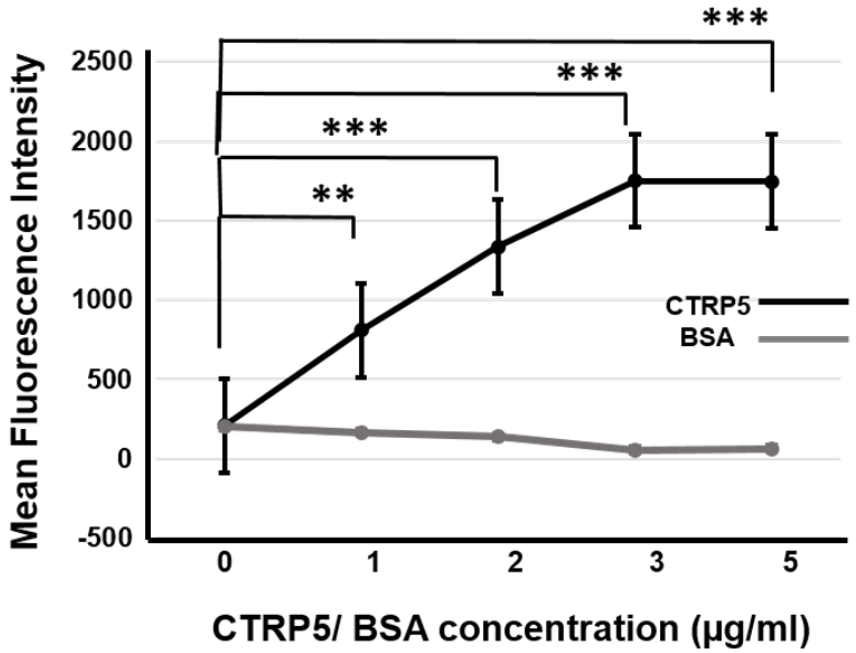
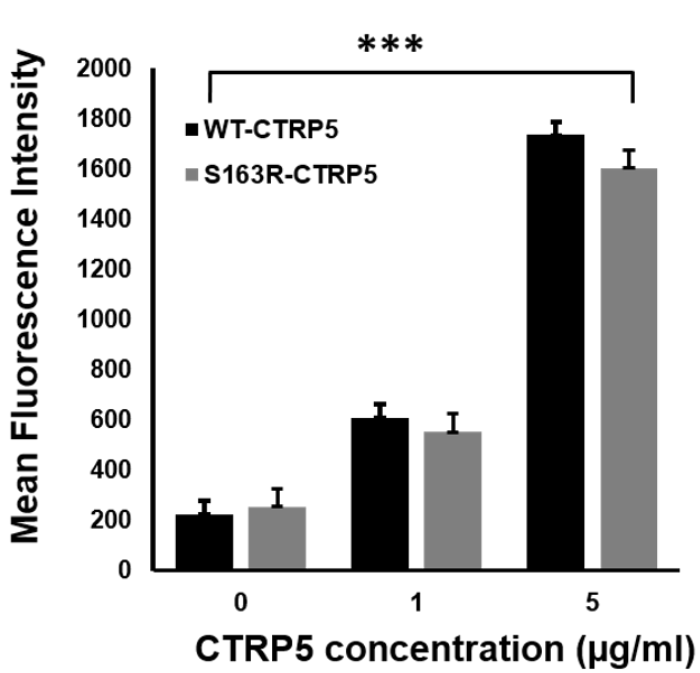

A
Collagen-like domain
C1q domain
PDZ ligand motif
WT-CTRP5 (1-243 aa)
CTRP5 ΔPDZ-ligand (1-238 aa)
B
SD-Leu-Trp
SD -Leu -Trp -His + 3-AT (2 mM)
AD-CL53 HTRA1
AD
AD-WT-CTRP5
BD- WT-CTRP5
BD
BD-CTRP5 ΔPDZ-Ligand
BD- WT-CTRP5
BD
BD-CTRP5 ΔPDZ-Ligand
C
PDZ domain of HTRA1
CTRP5
PDZ-binding motif of CTRP5

Figure 3:


***
***
***
**
Mean Fluorescence Intensity
2500
2000
1500
1000
500
0
-500
0
1
2
3
5
CTRP5
BSA
CTRP5/ BSA concentration (µg/ml)


B
***
WT-CTRP5
S163R-CTRP5
Mean Fluorescence Intensity
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
1
5
CTRP5 concentration (µg/ml)

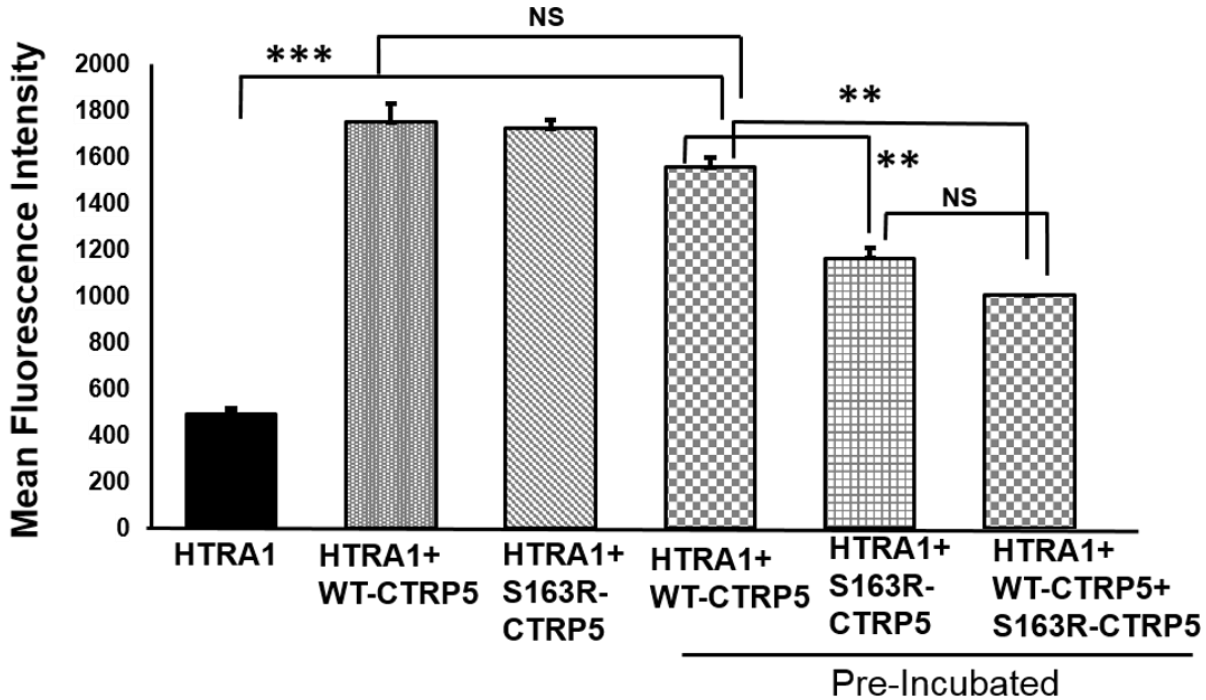
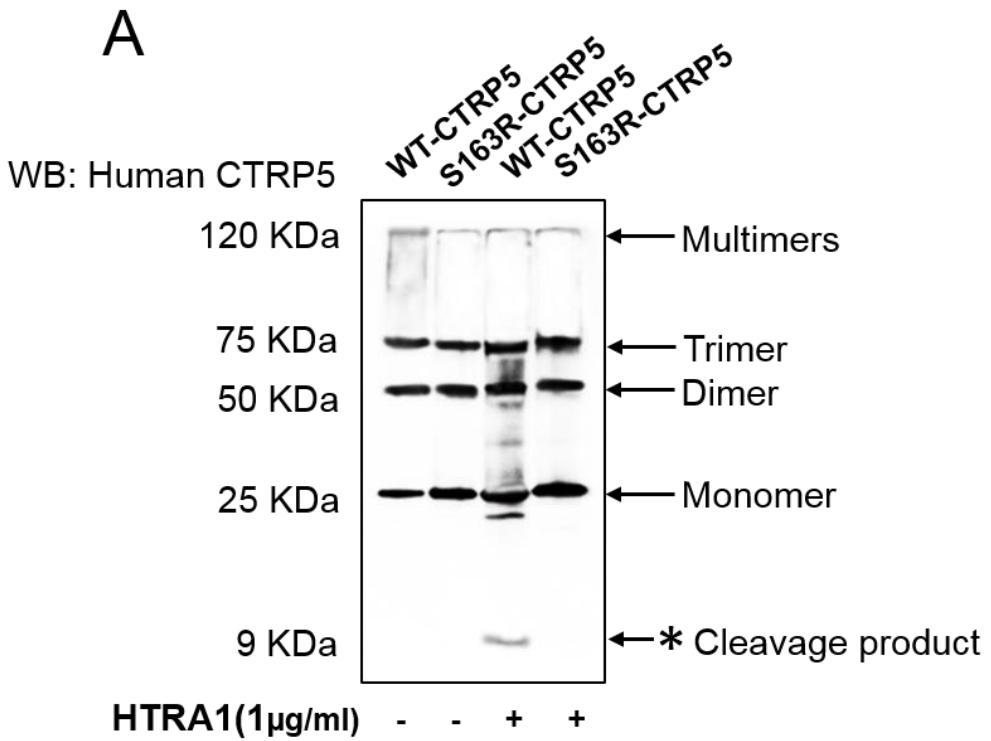
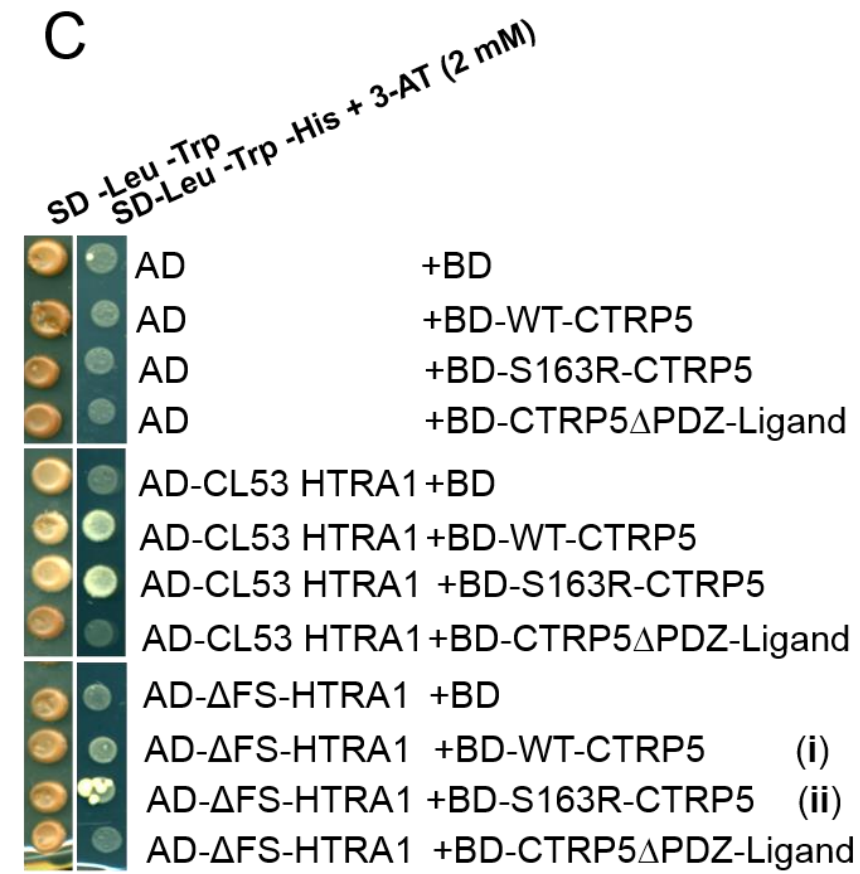
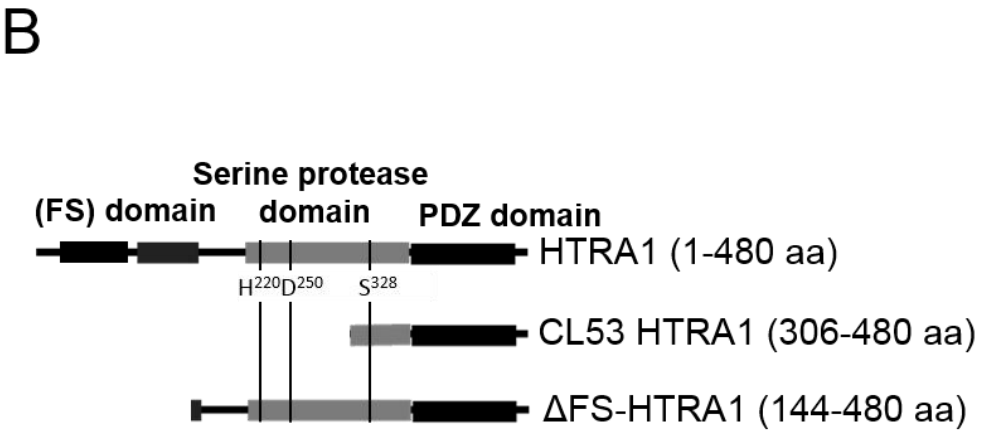

NS
***
**
**
NS
Mean Fluorescence Intensity
2000
1800
1600
1400
1200
1000
800
600
400
200
0
HTRA1
HTRA1+ WT-CTRP5
HTRA1+ S163R-CTRP5
HTRA1+ WT-CTRP5
HTRA1+ S163R-CTRP5
HTRA1+ WT-CTRP5+ S163R-CTRP5
Pre-Incubated

Figure 4:


A
WT-CTRP5
S163R-CTRP5
WT-CTRP5
S163R-CTRP5
WB: Human CTRP5
120 KDa
75 KDa
50 KDa
25 KDa
9 KDa
Multimers
Trimer
Dimer
Monomer
* Cleavage product
HTRA1(1µg/ml) - - + +


C
SD -Leu -Trp
SD-Leu -Trp -His + 3-AT (2 mM)
AD +BD
AD +BD-WT-CTRP5
AD +BD-S163R-CTRP5
AD +BD-CTRP5ΔPDZ-Ligand
AD-CL53 HTRA1 +BD
AD-CL53 HTRA1 +BD-WT-CTRP5
AD-CL53 HTRA1 +BD-S163R-CTRP5
AD-CL53 HTRA1 +BD-CTRP5ΔPDZ-Ligand
AD-ΔFS-HTRA1 +BD
AD-ΔFS-HTRA1 +BD-WT-CTRP5 (i)
AD-ΔFS-HTRA1 +BD-S163R-CTRP5 (ii)
AD-ΔFS-HTRA1 +BD-CTRP5ΔPDZ-Ligand


B
Serine protease
(FS) domain
domain
PDZ domain
HTRA1 (1-480 aa)
H220D250
S328
CL53 HTRA1 (306-480 aa)
ΔFS-HTRA1 (144-480 aa)

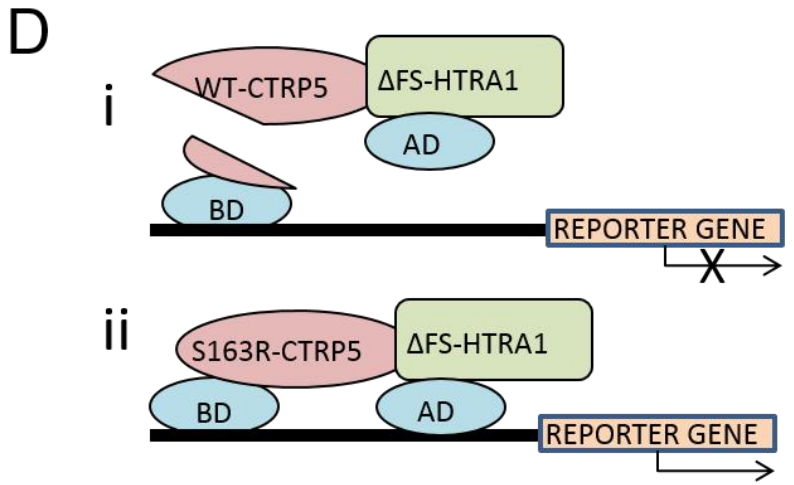

D
i
WT-CTRP5
ΔFS-HTRA1
AD
BD
REPORTER GENE
X
ii
S163R-CTRP5
ΔFS-HTRA1
BD
AD
REPORTER GENE

Figure 5:

A

Proteins	Mass intensities x 10^{-8}					
	Ctrp5$^{Wt/Wt}$ 5 mo	*Ctrp5*$^{S163R/Wt}$ 5 mo	*Ctrp5*$^{S163R/S163R}$ 5 mo	*Ctrp5*$^{Wt/Wt}$ 18 mo	*Ctrp5*$^{S163R/wt}$ 18 mo	*Ctrp5*$^{S163R/S163R}$ 18 mo
CTRP5	0.82 (0.1)	8.1 (0.9)	18.4 (1.7)	1.14 (0.2)	14 (4)	31 (4)
HTRA1	0.15 (0.04)	0.8 (0.07)	2.3 (0.05)	0.4 (0.08)	3 (0.2)	9.5 (0.2)

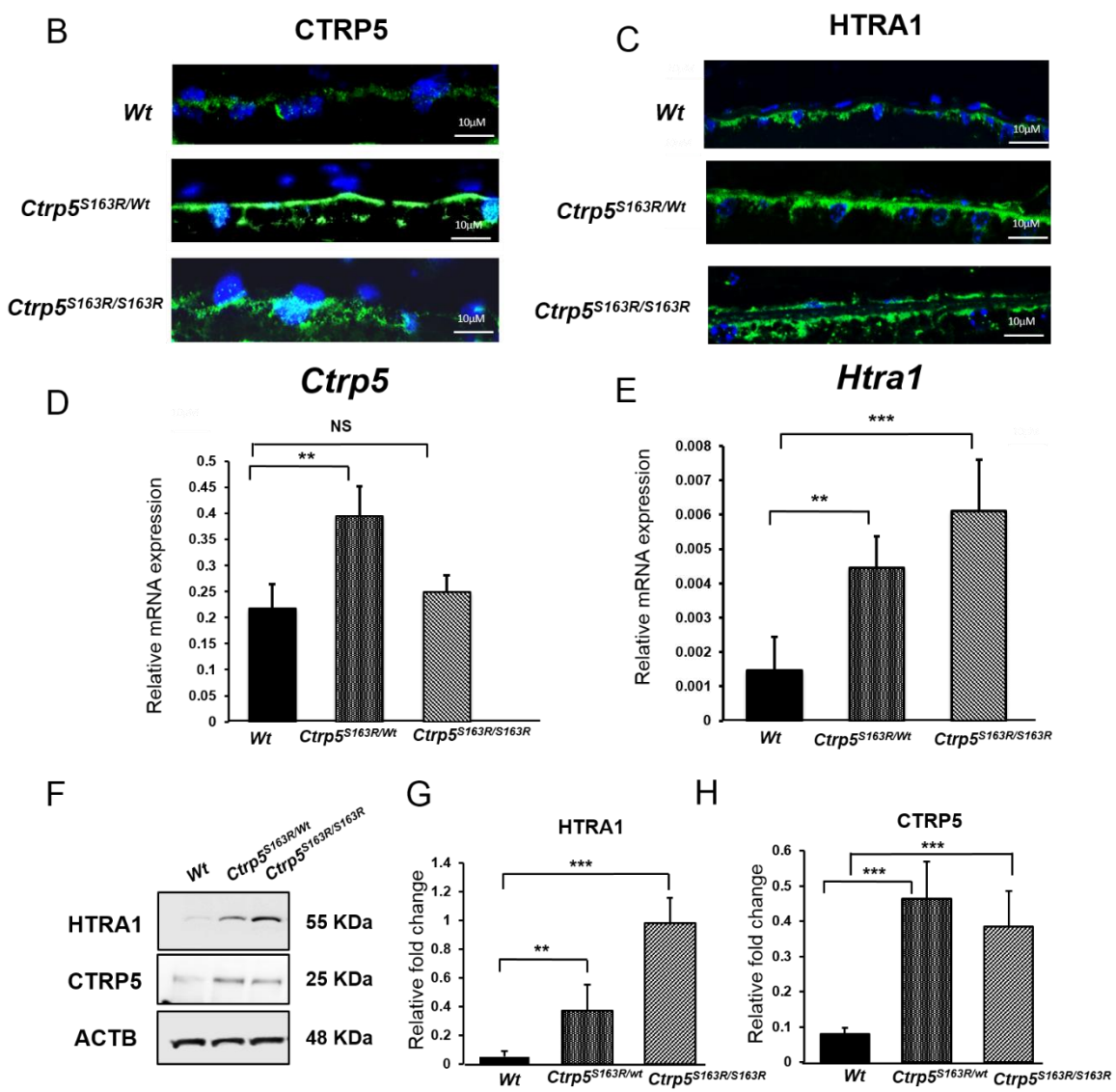
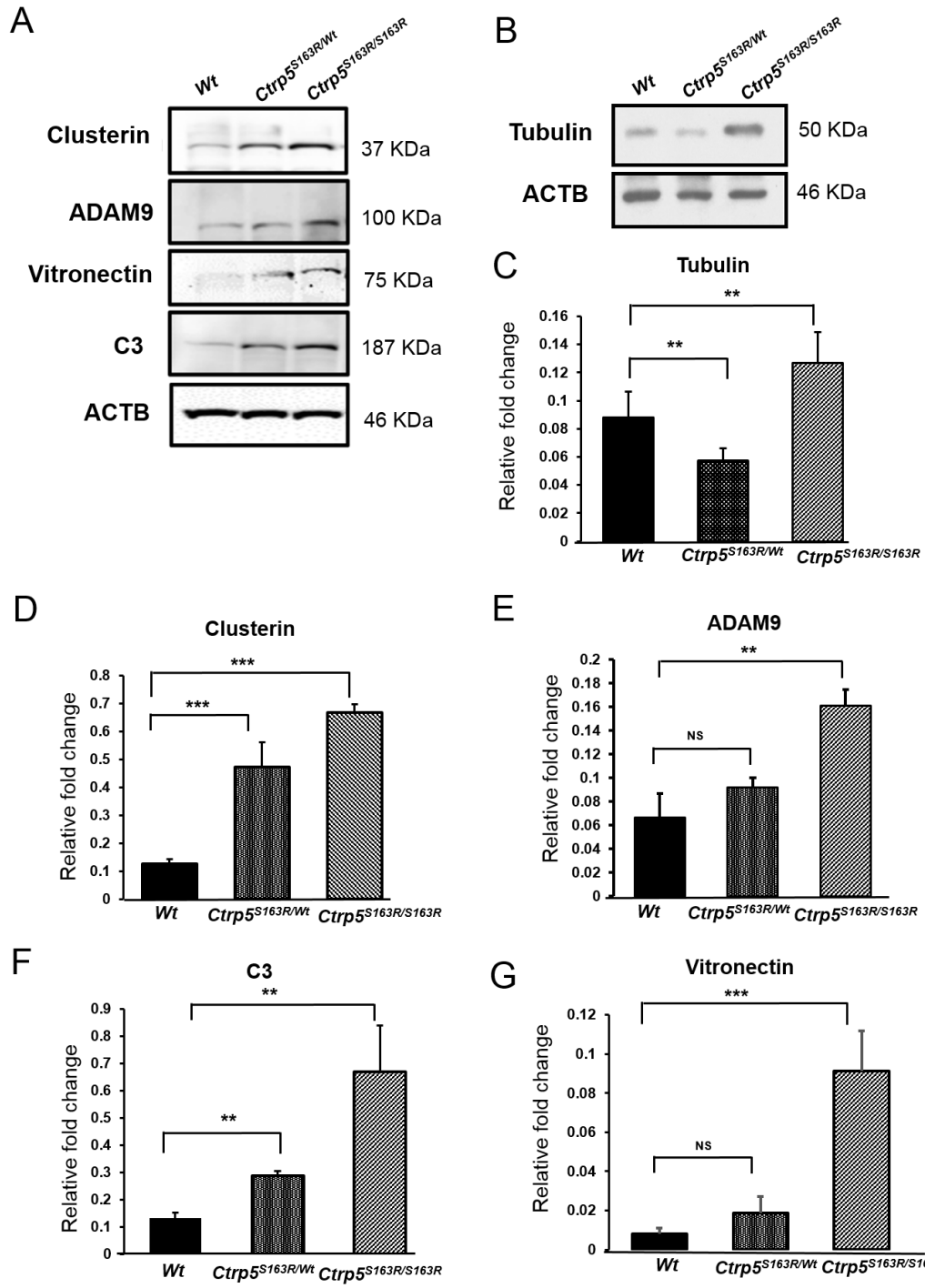
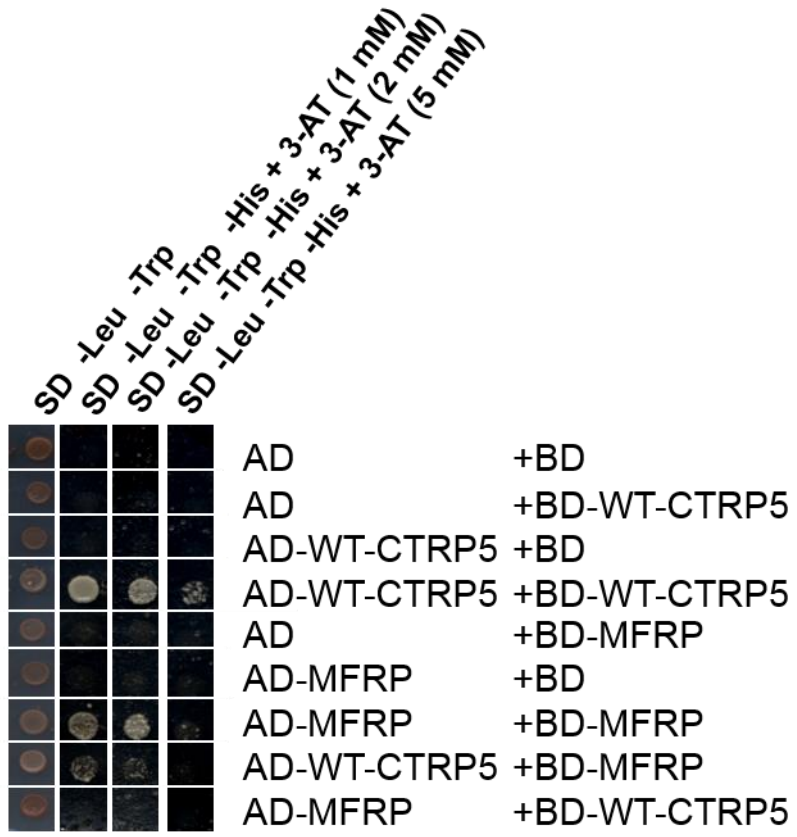


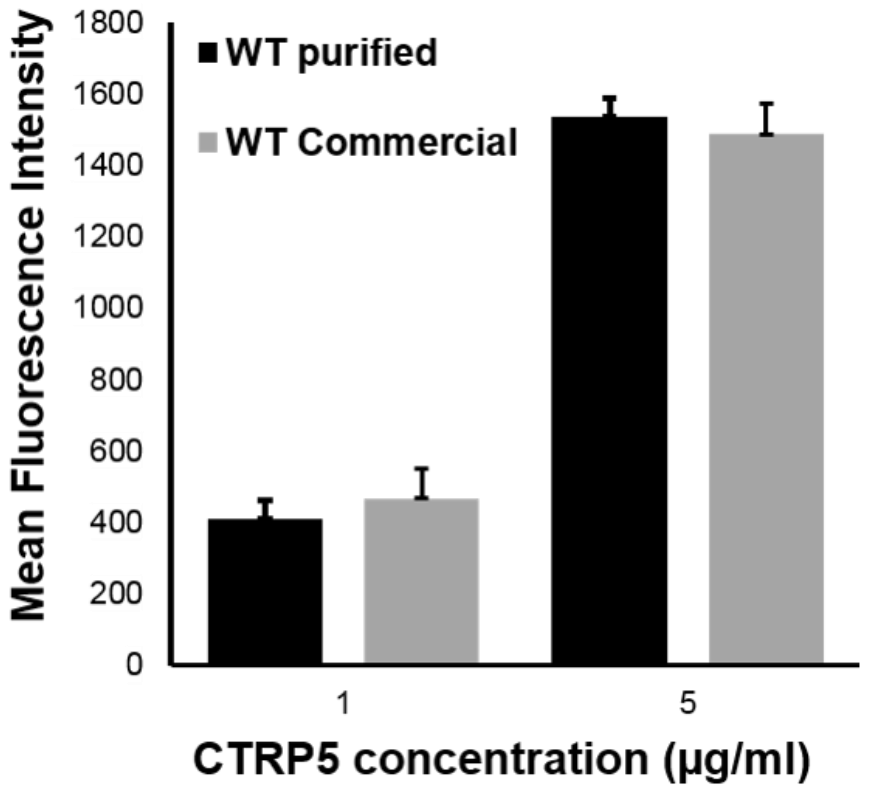
Figure 6:


A
Wt
Ctrp5S163R/Wt
Ctrp5S163R/S163R
Clusterin
37 KDa
ADAM9
100 KDa
Vitronectin
75 KDa
C3
187 KDa
ACTB
46 KDa
B
Wt
Ctrp5S163R/Wt
Ctrp5S163R/S163R
Tubulin
50 KDa
ACTB
46 KDa
C
Tubulin
Relative fold change
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
**
**
Wt
Ctrp5S163R/Wt
Ctrp5S163R/S163R
D
Clusterin
Relative fold change
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
***
***
Wt
Ctrp5S163R/Wt
Ctrp5S163R/S163R
E
ADAM9
Relative fold change
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
**
NS
Wt
Ctrp5S163R/Wt
Ctrp5S163R/S163R
F
C3
Relative fold change
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
**
**
Wt
Ctrp5S163R/Wt
Ctrp5S163R/S163R
G
Vitronectin
Relative fold change
0.12
0.1
0.08
0.06
0.04
0.02
0
***
NS
Wt
Ctrp5S163R/Wt
Ctrp5S163R/S163R

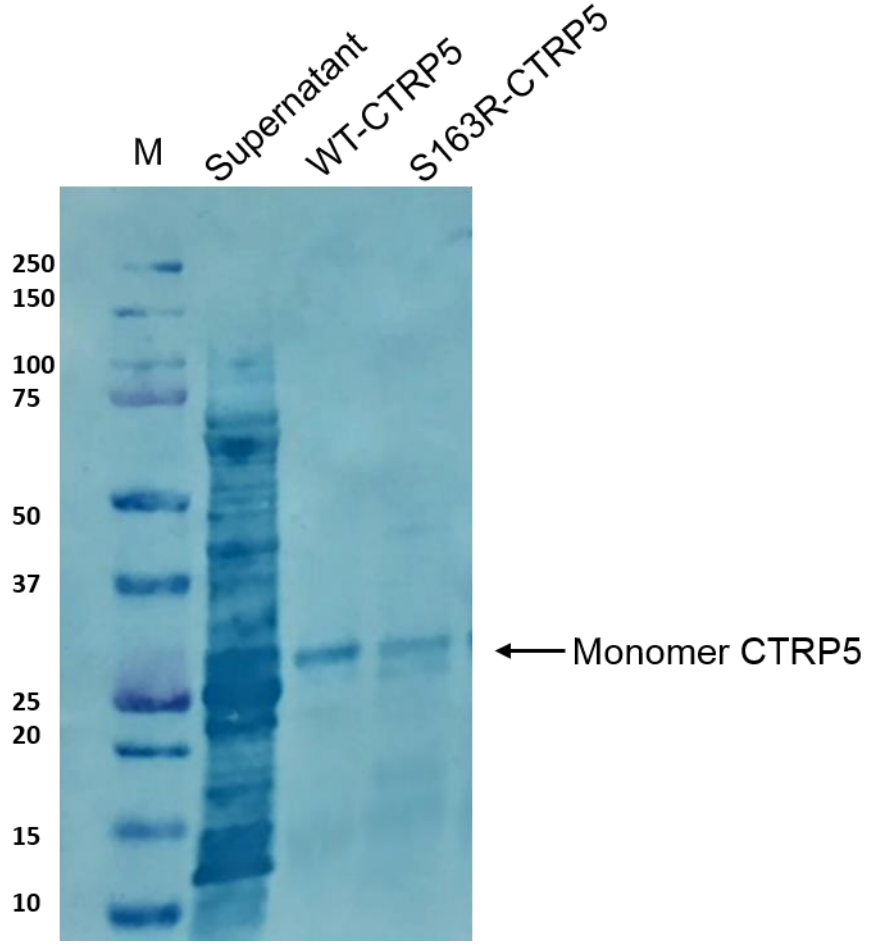
Supplementary Figure S1:



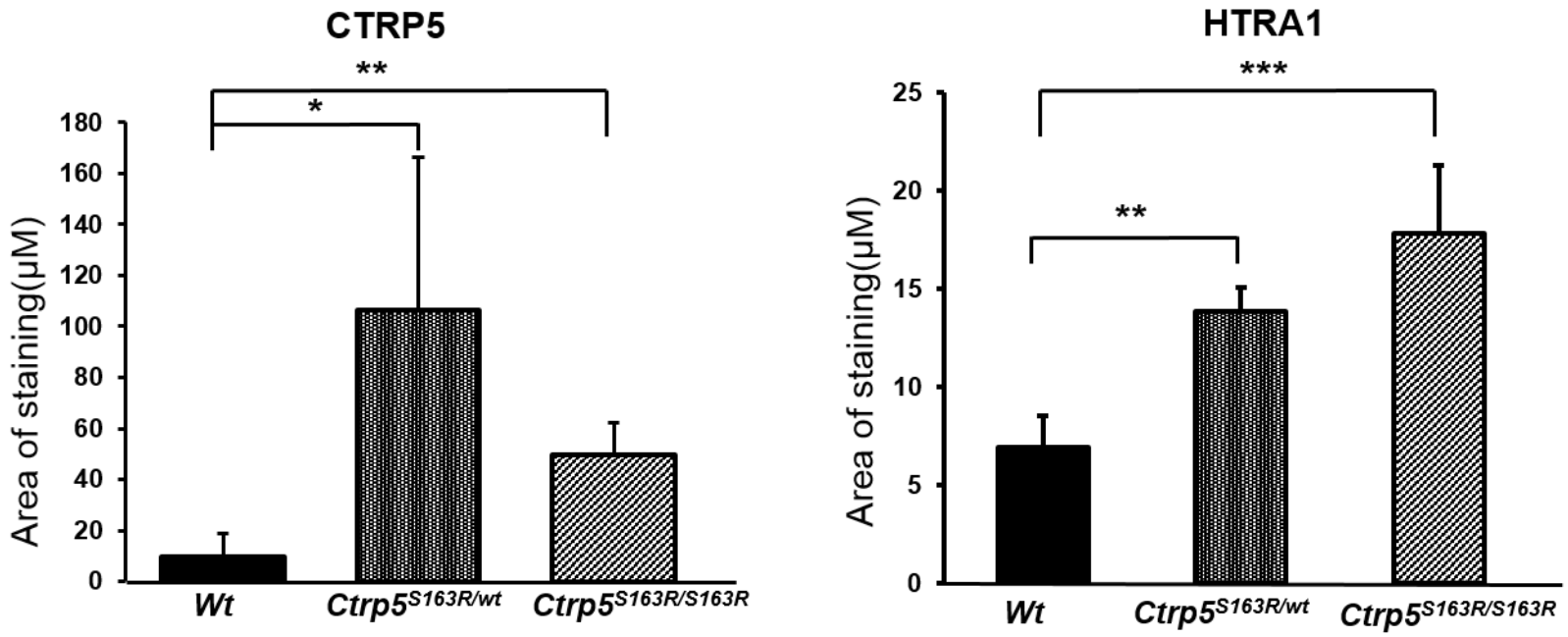
Supplementary Figure S2:



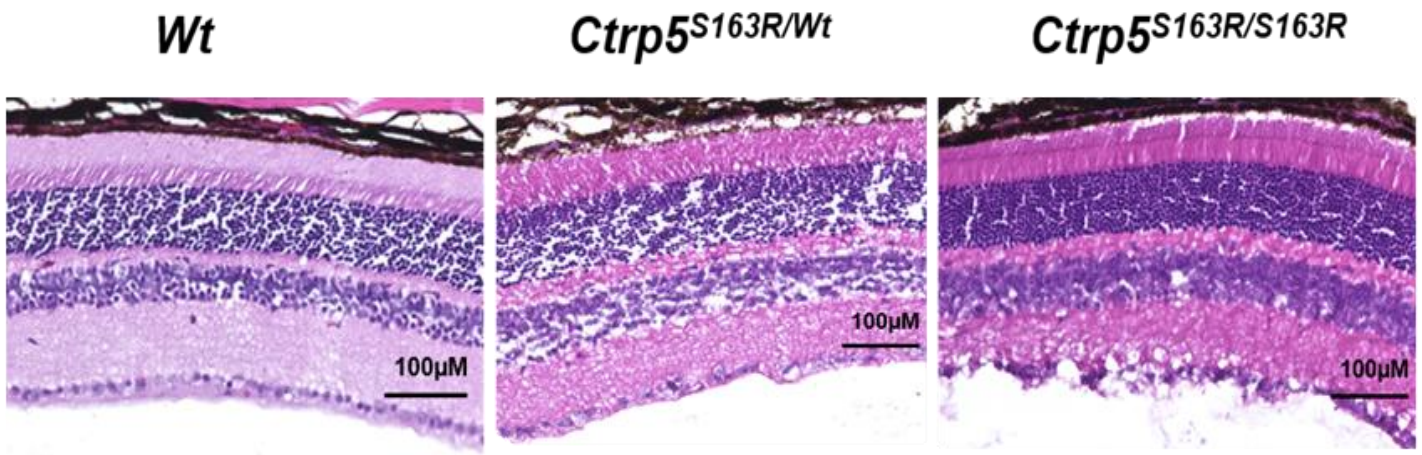
Supplementary Figure S3:



Supplementary Figure S4:



Supplementary Figure S5:



Supplementary table S1:

Saccharomyces cerevisiae strains					
Strain	Genotype				Reference
AH109	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::$GAL1_{UAS}$-$GAL1_{TATA}$-HIS3, $GAL2_{UAS}$-$GAL2_{TATA}$-ADE2, URA3::$MEL1_{UAS}$-$MEL1_{TATA}$-lacZ				TaKaRa
Y2HGold	MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::$GAL1_{UAS}$-$GAL1_{TATA}$-HIS3, $GAL2_{UAS}$-$Gal2_{TATA}$-ADE2 URA3::$MEL1_{UAS}$-$MEL1_{TATA}$ AUR1-C MEL1				TaKaRa
Plasmids for Y2H screening					
Plasmid name	AD-fusion protein	BD-fusion protein	Background	Selectable marker	Reference
pKZR042	WT-CTRP5 (1-243 aa)		pDEST-AD	Amp/Leu	this study
pKZR043		WT-CTRP5 (1-243 aa)	pDEST-BD	Kan/Trp	this study
pKZR044		WT-CTRP5 (1-243 aa)	pGBKT7	Kan/Trp	this study
pKZR045		S163R-CTRP5 (1-243 aa)	pGBKT7	Kan/Trp	this study
pKZR046	CL53 HTRA1 (306-480 aa)		pGADT7-RecAB	Amp/Leu	TaKaRa (Universal Human Normalized Mate & Plate™ Library)
pKZR047		WT-CTRP5 ΔPDZ-Ligand (1-238 aa)	pGBKT7	Kan/Trp	this study
pKZR048		MFRP	pDEST-BD	Kan/Trp	this study
pKZR049	MFRP		pDEST-AD	Amp/Leu	this study
pKZR050	ΔFS-HTRA1 (144-480 aa)		pGADT7	Amp/Leu	this study
Plasmids for protein expression in E.coli					
Plasmid name	Protein		Back ground	Selectable marker	Reference
pDEST14-C1QTNF5	WT-CTRP5-His		pDEST14	Amp	Stanton et al 2017
pDEST14-C1QTNF5-S163R	S163R-CTRP5-His		pDEST14	Amp	Stanton et al 2017

Supplementary table S2:

Mass intensities x 10^{-8}							
Peptide	Sequence	*Ctrp5*$^{Wt/Wt}$ 5 mo	*Ctrp5*$^{S163R/Wt}$ 5 mo	*Ctrp5*$^{S163R/S163R}$ 5 mo	*Ctrp5*$^{Wt/Wt}$ 18 mo	*Ctrp5*$^{S163R/Wt}$ 18 mo	*Ctrp5*$^{S163R/S163R}$ 18mo
128-142	VLLNEQGHYDPTTGK	1.3	4.9	8.2	1.4	10.0	21.0
162-170	ASLQFDLVK	0.5	0.6	0	0.4	1.2	0
164-170	LQFDLVK	0.1	1.8	2.1	0	2.1	6.5